

## CLAIMS

### WHAT IS CLAIMED IS:

1. A staining solution for detecting fusion proteins comprising an affinity tag, wherein  
5 said staining solution comprises:
  - a) a fluorescent compound capable of selectively binding, directly or indirectly, to  
said affinity tag, wherein said fluorescent compound comprises a fluorophore;  
and,
  - a. a buffer;10 with the proviso that the fluorescent compound does not comprise an antibody or  
fragment thereof.
2. The staining solution according to Claim 1, wherein said fluorescent compound is  
15 capable of selectively binding to a poly-histidine, GST, poly-arginine or Glu-Glu  
affinity tags.
3. The staining solution according to Claim 1, wherein said fluorescent compound is  
20 according to formula  $A(L)_m(B)_n$  wherein A is a fluorophore, L is a linker, B is binding  
domain, m is an integer from 1 to 4 and n is an integer from 1 to 6.
4. The staining solution according to Claim 3, wherein said fluorophore is selected from  
the group consisting of xanthene, coumarin, cyanine, acridine, anthracene,  
benzofuran, indole and borapolyazaindacene.
- 25 5. The staining solution according to Claim 4, wherein said fluorescent compound  
comprises glutathione as a binding domain and xanthene as a fluorophore.
6. The staining solution according to Claim 4, wherein said binding domain is an acetic  
acid binding domain.
- 30 7. The staining solution according to Claim 6, wherein said acetic acid binding domain  
is capable of selectively binding, directly or indirectly, to a poly-histidine or a poly-  
arginine affinity tag
- 35 8. A staining solution for detecting fusion proteins comprising a poly-histidine affinity  
tag, wherein said staining solution comprises:
  - a) a fluorescent compound having formula  $A(L)_m(B)_n$  wherein A is a

fluorophore, L is a linker, B is an acetic acid binding domain capable of selectively binding to a poly-histidine affinity tag, m is an integer from 1 to 4 and n is an integer from 1 to 6; and,

- b) a buffer having a pH of about 5 to 6.9 and comprising an acceptable counter ion

with the proviso that said binding domain does not comprise an antibody or fragment thereof.

9. The staining solution according to Claim 8, wherein said buffer comprises a salt.

10. The staining solution according to Claim 9, wherein said fluorophore is selected from the group consisting of xanthene, coumarin, cyanine, acridine, anthracene, benzofuran, indole and borapolyazaindacene.

11. The staining solution according to Claim 10, wherein said buffer has a pH of about 6.5.

12. The staining solution according to Claim 11, wherein said buffer further comprises a metal ion selected from the group consisting of nickel and cobalt.

13. The staining solution according to Claim 12, wherein said staining solution comprises nickel ions at a final concentration of about 1  $\mu$ M to 150  $\mu$ M.

14. A method for selectively detecting an affinity tag containing fusion protein in a sample, said method comprising the steps of:

- a) contacting said sample with a staining solution according to any one of Claims 1-13; and,  
b) illuminating said fluorescent compound whereby said fusion protein is detected with the proviso that said fluorescent compound does not comprise an antibody or fragment thereof.

15. The method according to Claim 14, wherein said method further comprises first immobilizing said sample on a solid or semi-solid matrix.

16. The method according to Claim 14, wherein said affinity tag is selected from the group consisting of poly-histidine, GST, poly-arginine and Glu-Glu affinity tags.

17. The method according to Claim 16, wherein said fluorophore is selected from the group consisting of a xanthene, coumarin, cyanine, acridine, anthracene, benzofuran, indole and borapolyazaindacene.
- 5 18. The method according to Claim 17, wherein said compound comprises formula  $A(B)_n$  wherein A is a fluorophore, B is a binding domain that is a chemical moiety, protein or fragment thereof capable of selectively binding said affinity tag and n is an integer from 1 to 6.
- 10 19. The method according to Claim 18, wherein said chemical moiety is an acetic acid binding domain.
20. The method according to Claim 19, wherein said buffer further comprises an indirect binding reagent capable of forming a complex between said affinity peptide and said binding moiety.
- 15 21. A method for detecting a poly-histidine affinity tag containing fusion protein in a sample, said method comprising the steps of:
- 20 i) immobilizing said sample on a solid or semi-solid matrix;
- ii) optionally contacting said sample of step i) with a fixing solution;
- iii) contacting said sample of step i) or ii) with a staining solution according to any one of Claims 7-13;
- iv) incubating said staining solution and said sample for sufficient time to allow said compound to associate either directly or indirectly with said poly-histidine affinity tag;
- 25 v) illuminating fluorophore of said staining solution with a suitable light source whereby said fusion protein is detected.
22. The method according to Claim 21, wherein said buffer has a pH of about 6.5.
- 30 23. The method according to Claim 22, wherein said buffer comprises a salt.
24. The method according to Claim 23, wherein said buffer has a pKa of about 6.0 to about 7.5.
- 35 25. The method according to Claim 24, wherein said fluorophore is selected from the group consisting of xanthene, cyanine, coumarin, acridine, anthracene, benzofuran,

borapolyazaindacene and derivative thereof.

26. The method according to Claim 25, wherein fluorescent compound of said staining solution comprises at least three acetic acid groups.

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27. The method according to Claim 26, wherein immobilizing said sample comprises electrophoretically separating on a polymeric gel.

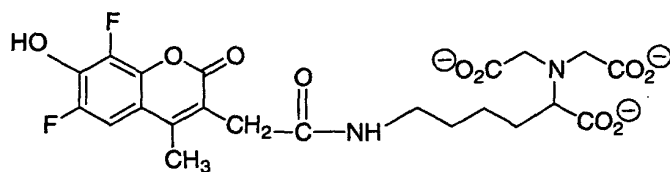
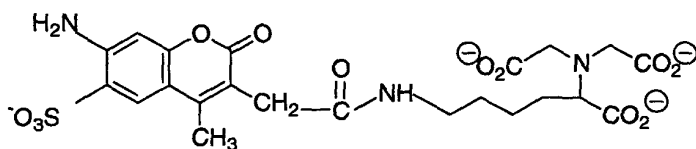
28. The method according to Claim 27, wherein said fixing solution comprises an alcohol.

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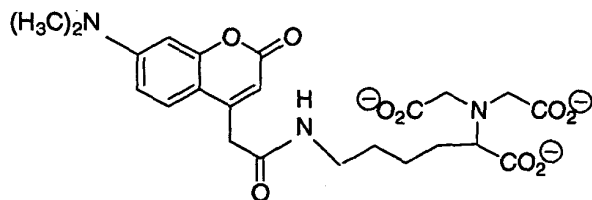
29. The method according to Claim 28, wherein said method further comprises contacting said gel with a total protein stain.

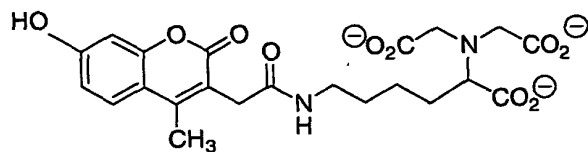
30. The method according to Claim 27, wherein said fluorophore is a coumarin and said compound is selected from the group consisting of

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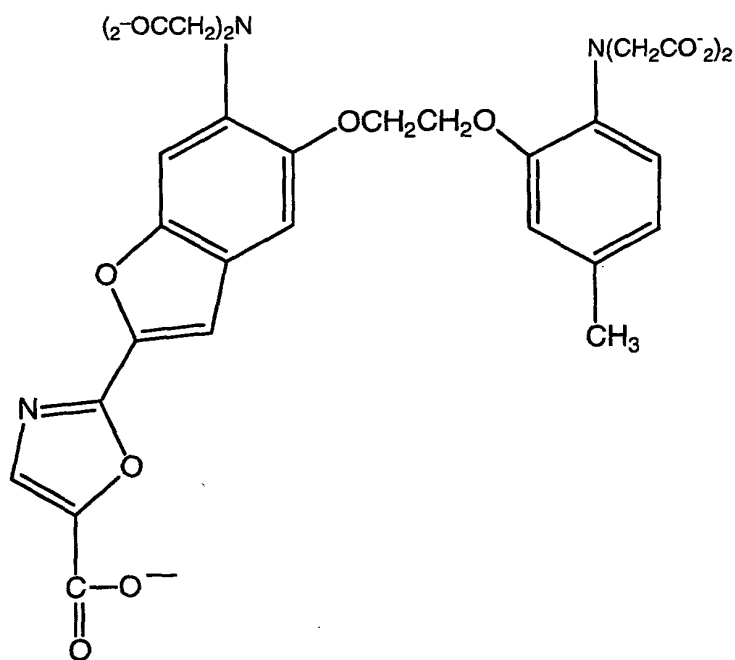
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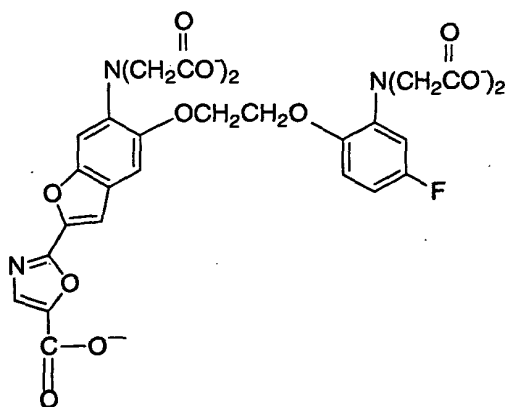


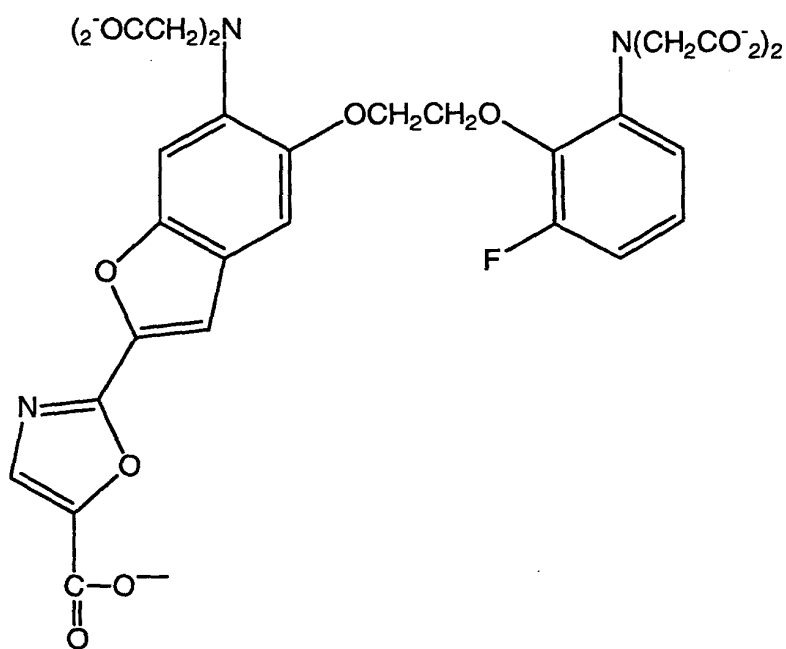
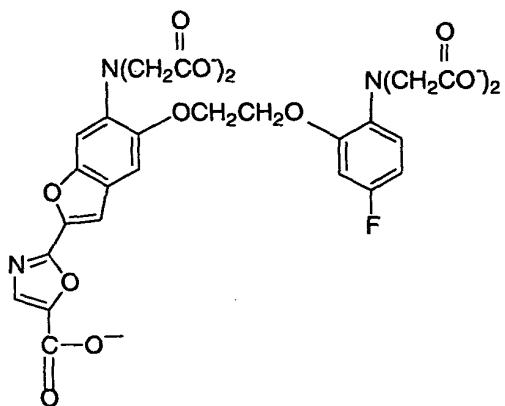
and salts thereof.

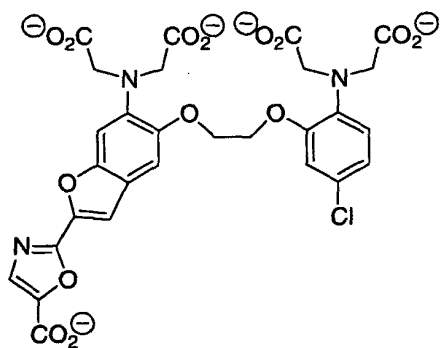
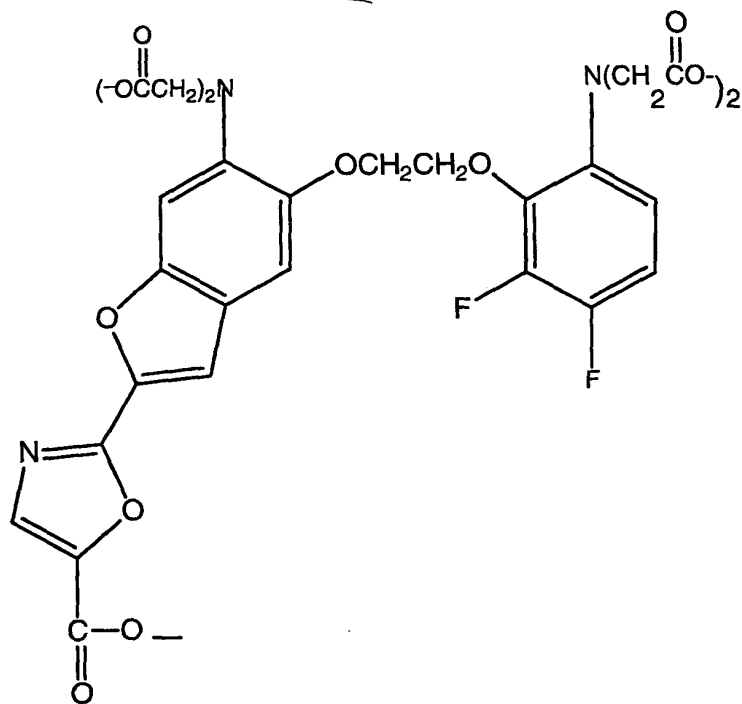
- 5 31. The method according to Claim 27, wherein said fluorophore is a benzofuran and said compound is selected from the group consisting of



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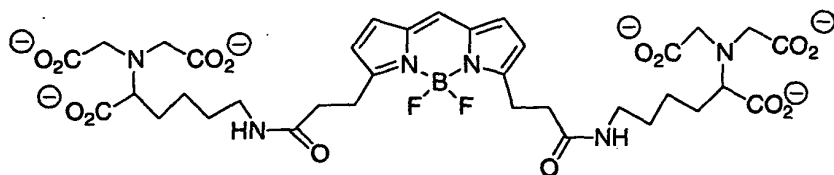


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and salts thereof.

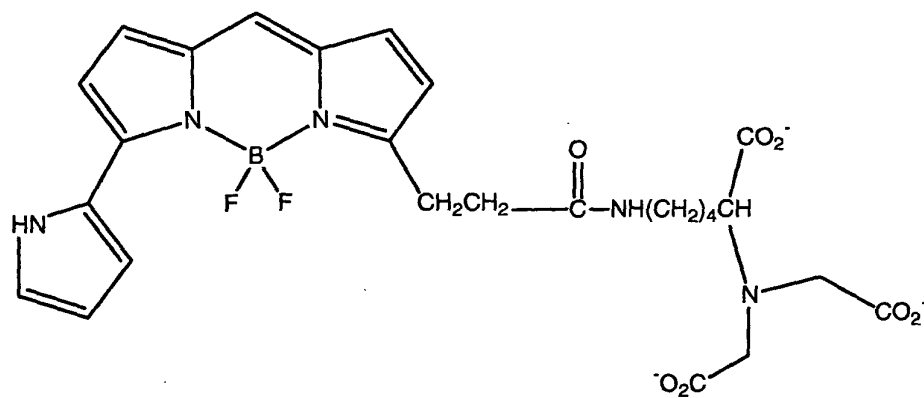
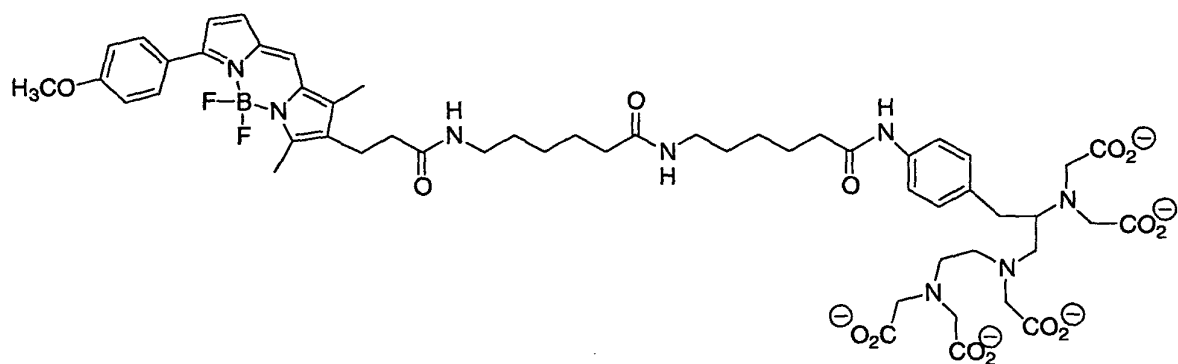
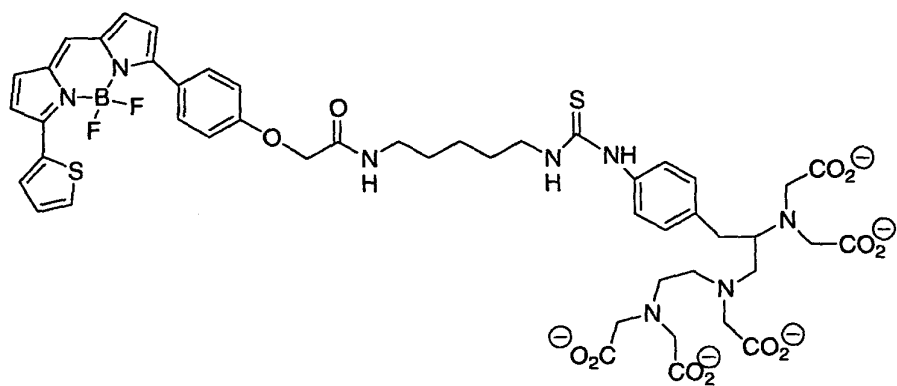
32. The method according to Claim 27, wherein said fluorophore is a borapolyazaindacene and said compound is selected from the group consisting of

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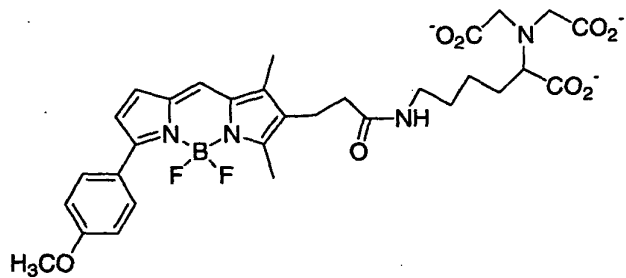


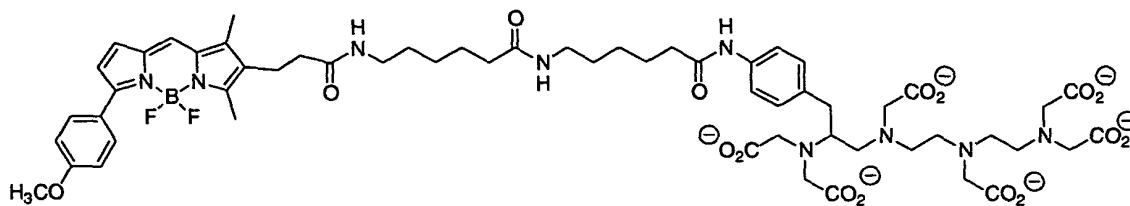






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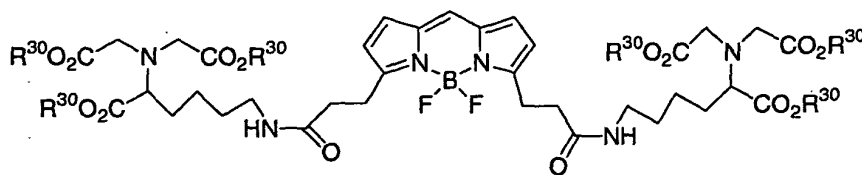


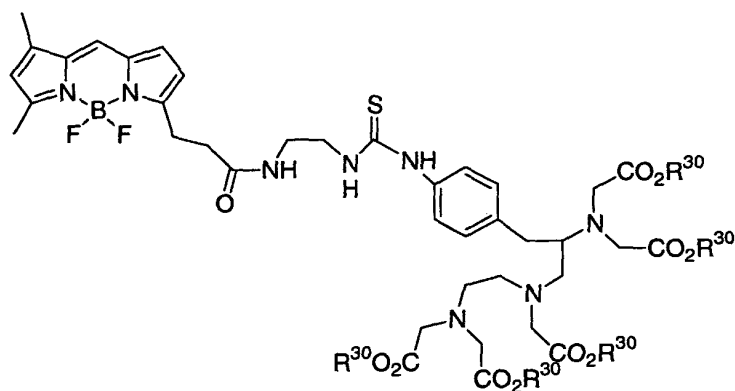
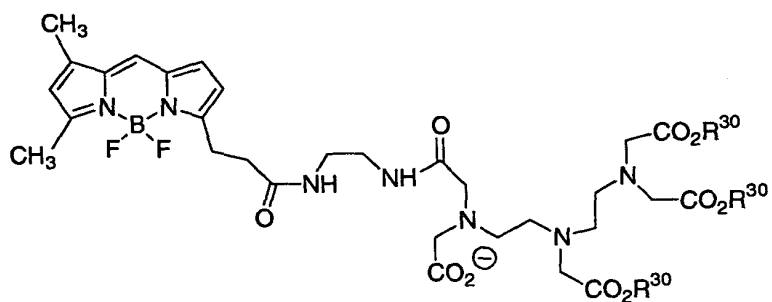


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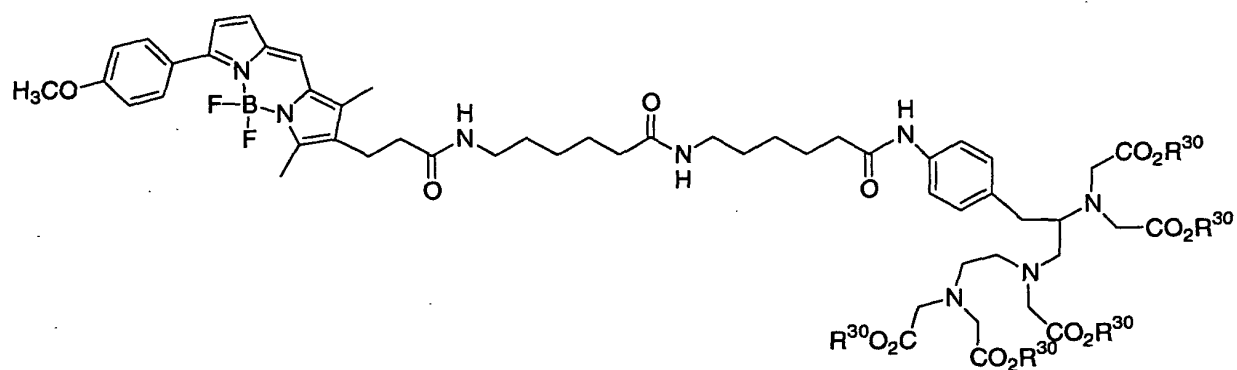
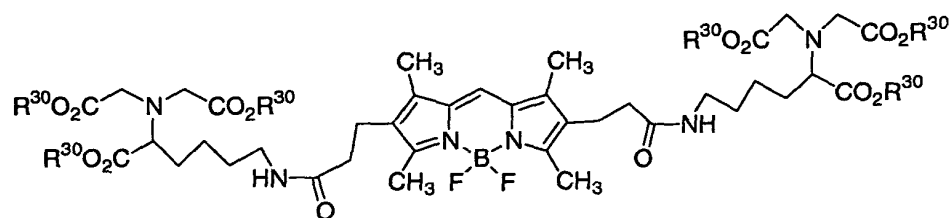
- 5 33. The method according to any one of Claims 30, 31 or 32, wherein said compound binds directly to said affinity tag of said fusion protein.
34. The method according to any one of Claims 30, 31 or 32, wherein said buffer further comprises a metal ion and said compound indirectly binds said affinity tag by forming a ternary complex.
- 10 35. The method according to Claim 34 wherein said metal ion is nickel or cobalt.
36. A kit for detecting an affinity tag containing fusion protein, wherein said kit comprises; a staining solution according to anyone of Claims 1-13 comprising a fluorescent compound and a buffer with the proviso that the fluorescent compound does not comprise an antibody or fragment thereof.
- 15 37. The kit according to Claim 36, wherein said kit further comprises, alone or in combination, molecular weight markers, fixing solution, wash solution and an additional detection reagent.
- 20 38. The kit according to Claim 36, wherein said additional detection reagent is a total protein stain.
- 25 39. The kit according to Claim 36, wherein said fluorescent compound comprises a binding domain and a fluorophore selected from the group consisting of a xanthene, cyanine, coumarin, acridine, anthracene, benzofuran, borapolyazaindacene and derivative thereof.
- 30 40. The kit according to Claim 39, wherein said fluorescent compound is according to formula A(L)m(B)n wherein A is a fluorophore, L is a linker, B is an acetic acid binding domain, m is an integer from about 1 to 4 and n is an integer from about 1 to 6 wherein said fluorescent compound comprises at least three acetic acid groups.

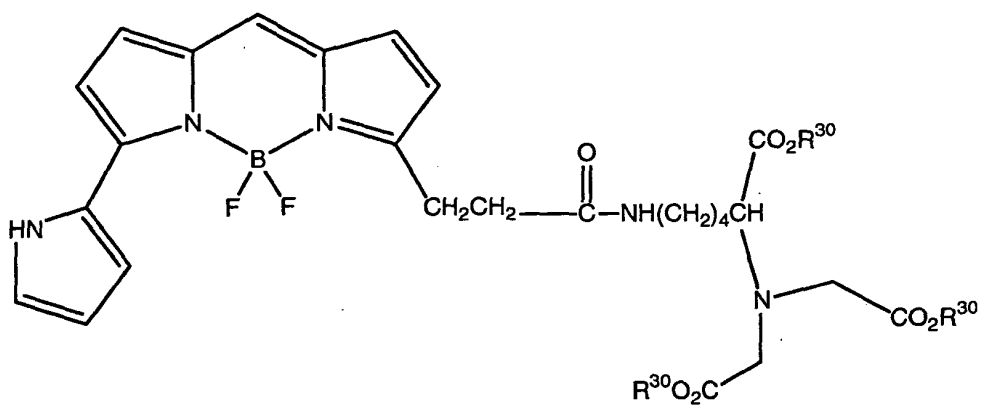
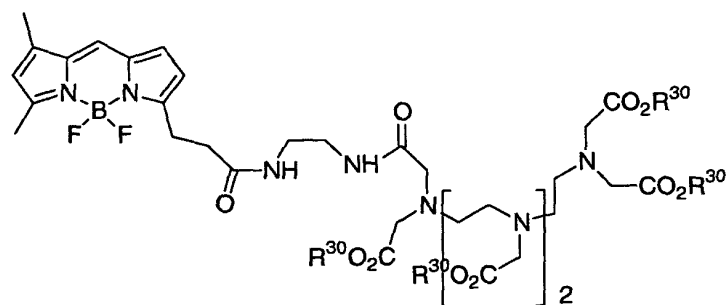
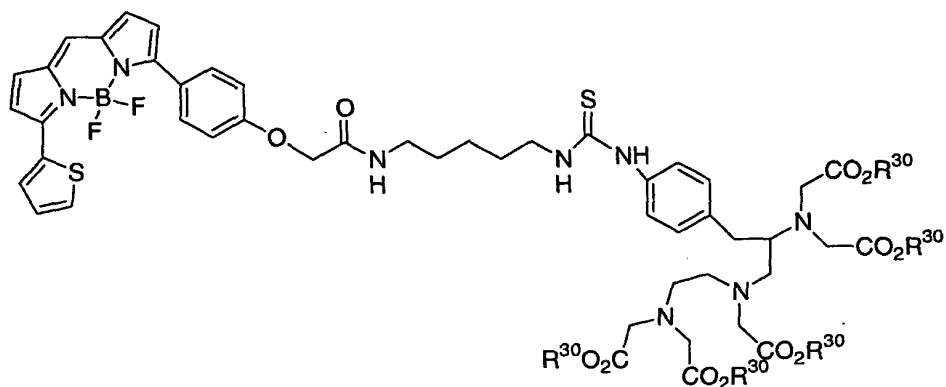
41. The kit according to Claim 40 wherein said buffer has a pH between about 5 to about 6.9 and said buffer optionally comprises a metal ion selected from the group consisting of nickel and cobalt.
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42. The kit according to Claim 39, wherein said binding domain is glutathione.
43. A fluorescent compound having formula  $A(L)_m(B)_n$ , wherein A is a fluorophore selected from the group consisting of borapolyazaindacene and coumarin, L is a linker, B is an acetic acid binding domain wherein said fluorescent compound contains at least three acetic acid groups that are capable of binding to a poly-histidine affinity tag, m is an integer from 1 to 4 and n is an integer from 1 to 6.
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44. The compound according to Claim 43, wherein said linker is selected from the group consisting of  $-(CH_2)_eC(X)NH(CH_2)_e(NHC(X)(CH_2)_e)_d-$ ,  
 .....  $-((C_6R''_4)O)_d(CH_2)_e(C(X)NH(CH_2)_e)(NH)_dC(X)NH(C_6R''_4)(CH_2)_e-$  and  
 $-(O)_d(CH_2)_fO(C_6R''_4)-$  wherein X is O or S, d is 0 or 1, e is 1 to 6, f is 2 or 3, and R'' is independently H, halogen, alkyl or alkyl.
- 15
45. The compound according to Claim 44, wherein said acetic acid binding domain is selected from the group consisting of  $O_2CCH(R)N(CH_2CO_2^-)_2$ ,  $-N(CH_2CO_2)_2$  and  $(CH_2CO_2^-)_ZN[(CH(R))_SN(CH_2CO_2^-)]_T(CH(R))_SN(CH_2CO_2^-)_Z$  wherein Z is 1 or 2, S is 1 to 5, T is 0 to 4 and R is said linker.
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46. The compound according to Claim 45, wherein said fluorophore is a borapolyazaindacene and said compound is selected from the group consisting of
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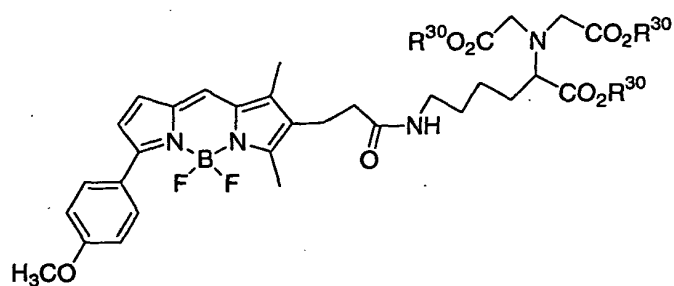


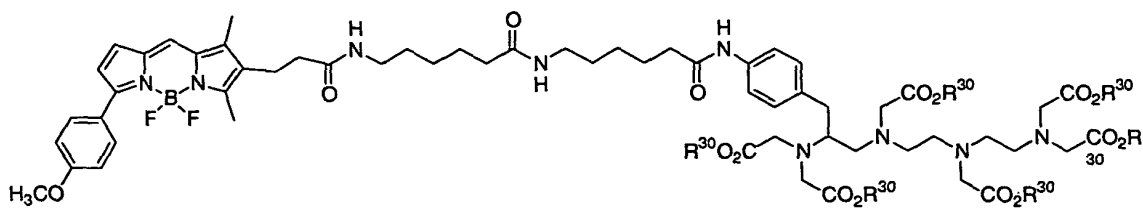
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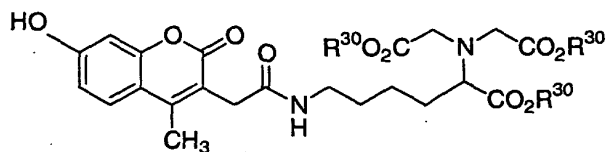
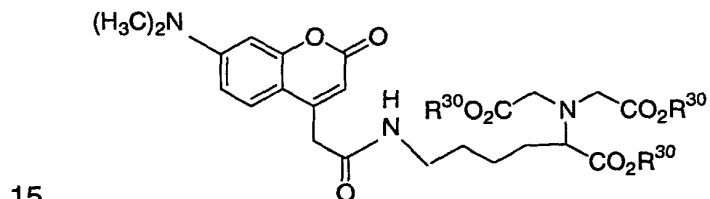
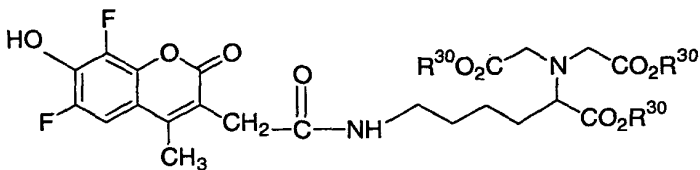
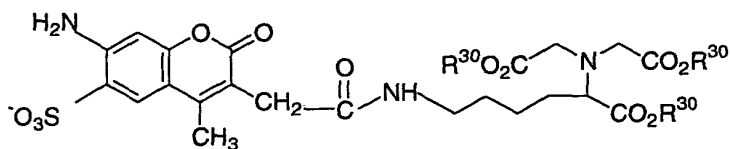
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and salts thereof wherein  $R^{30}$  may be the same or different and is selected from the group  
 5 consisting of hydrogen, salt ion,  $-\text{CH}_2\text{OCOR}^{41}$  and an electron pair wherein  $R^{41}$  is an alkyl group.

47. The compound according to Claim 45, wherein said fluorophore is a coumarin and  
 10 said compound is selected from the group consisting of



and salts thereof wherein  $R^{30}$  may be the same or different and is selected from the group  
 20 consisting of hydrogen, salt ion,  $-\text{CH}_2\text{OCOR}^{41}$  and an electron pair wherein  $R^{41}$  is an alkyl group.

48. A composition comprising;
- 5 a) a fluorescent compound capable of selectively binding, directly or indirectly, to affinity tag containing fusion protein, wherein said fluorescent compound comprises a fluorophore; and,
- b) a fusion protein comprising an affinity tag, provided said fluorescent compound does not comprise an antibody or fragment thereof.
- 10 49. The composition according to Claim 48, wherein said fluorescent compound comprises a binding domain and a fluorophore selected from the group consisting of xanthene, cyanine, coumarin, acridine, anthracene, benzofuran, borapolyazaindacene and derivative thereof.
- 15 50. The composition according to Claim 49 wherein said fluorescent compound is according to formula  $A(L)_m(B)_n$  wherein A is a fluorophore, B is an acetic acid binding domain wherein said compound comprises at least three acetic acid groups that are capable of selectively binding to a poly-histidine affinity tag, m is an integer from 1 to 4 and n is an integer from 1 to 6.
- 20 51. The composition according to Claim 50, wherein said composition further comprises a metal ion selected from the group consisting of nickel and cobalt.
52. The composition according to Claim 49, wherein said binding domain is glutathione.
- 25